

Alaska Department of Fish and Game
Division of Wildlife Conservation
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Molecular Genetic Approaches in Wildlife Management

Kris J. Hundertmark

Research Performance Report
1 July 2001–30 June 2002
Federal Aid in Wildlife Restoration
Grants W-27-5
Project 1.54

This is a progress report on continuing research. Information may be refined at a later date.

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**FEDERAL AID
ANNUAL RESEARCH PERFORMANCE REPORT**

ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 25526
Juneau, AK 99802-5526

PROJECT TITLE: Molecular genetic approaches in wildlife management

PRINCIPAL INVESTIGATOR: Kris J. Hundertmark

COOPERATORS: Larry Van Daele

FEDERAL AID GRANT PROGRAM: Wildlife Restoration

GRANT AND SEGMENT NR.: W-27-5

PROJECT NR.: 1.54

WORK LOCATION: Kenai Moose Research Center, Soldotna

STATE: Alaska

PERIOD: 1 July 2001 – 30 June 2002

I. PROGRESS ON PROJECT OBJECTIVES

OBJECTIVE 1: Create and maintain a permanent frozen tissue bank and associated database.

We continued to add samples to the collection, particularly black bears from GMUs 1A and 2, and moose from Kalgin Island.

OBJECTIVE 2: Determine suites of nuclear and/or mitochondrial markers suitable for analysis at the individual and population levels for selected species.

A suite of microsatellite markers was tested for suitability of describing variability at the population level.

OBJECTIVE 3: Document genetic variation within and among populations of Alaskan wildlife for forensic and biological purposes.

Samples were submitted to an independent laboratory for analysis of genetic variation in the insular populations of Kalgin Island and Berners Bay.

OBJECTIVE 4: Develop both field and laboratory methods appropriate to conduct molecular-based CMR population analyses.

No progress was made toward this objective during this period.

II. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN THIS PERIOD

JOB 1: Preparation of study plan

JOB 2: Genetics technique development

In conjunction with activities relating to Job 4, we tested a suite of microsatellite markers for use with moose. These markers were: BL42, BM203, BM1225, BM4513, BM848, BM888, FCB193, Rt1, Rt5, Rt9, Rt24, and Rt30, and were originally described in bovine or reindeer genomes. At this writing, I can report success with amplification of these markers but the contract lab (Wildlife Genetics International, Nelson, British Columbia) is still attempting to maximize performance through changes in parameters of the polymerase chain reaction.

JOB 3: Caribou population genetics

No work was conducted during this period.

JOB 4: Moose population genetics

Tissue samples from 21 moose from Kalgin Island (GMU 16) and 8 moose from Berners Bay (GMU 1) were sent for analysis to Wildlife Genetics International, Nelson, British Columbia. Laboratory analysis was completed in late June but the data have not been reviewed or analyzed yet.

JOB 5: Brown bear population genetics

This job was not active.

JOB 6: Afognak elk genetics

Work proceeded on a publication in collaboration with L. Van Daele describing the results from the FY01 annual report. The manuscript will be submitted either to *Conservation Genetics* or to *Journal of Wildlife Management*. Also, a presentation was prepared for the 2001 Arctic Science Conference that was cancelled due to the national emergency of 9/11. The abstract of that presentation is attached as an appendix.

JOB 7: Prepare annual and final reports.

This annual report was prepared.

III. ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

Worked on preparing or revising the following journal publications from a previous federal aid project concerning moose genetics and a collaboration with R. T. Bowyer, University of Alaska Fairbanks.

- Hundertmark, K. J., R. T. Bowyer, G. F. Shields, C. C. Schwartz, and M. H. Smith. In review. Spatial variation in mtDNA haplotypes from moose in Alaska and northwestern British Columbia: taxonomic and management implications. *Canadian Journal of Zoology*.
- Bowyer, R. T., K. M. Stewart, B. M. Pierce, K. J. Hundertmark, and W. C. Gasaway. In press. Geographical variation in antler morphology of Alaskan moose: putative effects of habitat and genetics. *Alces*.
- Hundertmark, K. J., G. F. Shields, R. T. Bowyer, and C. C. Schwartz. In press. Genetic relationships deduced from cytochrome-*b* sequences among moose. *Alces*.
- Hundertmark, K. J., R. T. Bowyer, G. F. Shields, and C. C. Schwartz. Accepted pending revision. Mitochondrial phylogeography of moose (*Alces alces*) in North America. *Journal of Mammalogy*.
- Hundertmark, K. J., G. F. Shields, I. G. Udina, R. T. Bowyer, A. A. Danilkin, and C. C. Schwartz. 2002. Mitochondrial phylogeography of moose (*Alces alces*): late Pleistocene divergence and population expansion. *Molecular Phylogenetics and Evolution* 22:375-387.

A literature review was conducted concerning genetic variability of beluga whales in North America in order to brief Region II staff on the use of genetic data in efforts to classify the Cook Inlet population of belugas as threatened or endangered. That analysis was presented at the annual regional meeting in December, 2002. A memorandum was produced and is attached as an appendix.

IV. PUBLICATIONS

Hundertmark, K. J., and L. J. Van Daele. In prep. Founder effect in an insular, translocated population of elk (*Cervus elaphus*)

V. RECOMMENDATIONS FOR THIS PROJECT

None

VI. APPENDIX

Appendix 1. Abstract of presentation prepared for the 2001 Arctic Science Conference.

Founder Effect in a Translocated Population of Elk

Kris J. Hundertmark (Alaska Dept. of Fish and Game, Division of Wildlife Conservation, Soldotna, AK 99669; email kris_hundertmark@fishgame.state.ak.us)

Larry Van Daele (Alaska Dept. of Fish and Game, Division of Wildlife Conservation, Kodiak, AK, 99615; email larry_vandaele@fishgame.state.ak.us)

Two fundamental concerns of conservation biology are the viability of small populations and the reintroduction of endangered species to their former ranges. As both topics are intertwined with population genetics, important lessons can be learned from examination of genetic characteristics of populations that experienced a documented reduction of effective population size through either a bottleneck or founder event. We assessed genetic variability in such a population in

Alaska: elk (*Cervus elaphus roosevelti*) of the Kodiak Archipelago. The initial transplant involved eight yearlings (three males, five females) from the Olympic Peninsula, Washington in 1929; in the interim, no additional transplants have been documented and the population is isolated from all sources of immigration. The current population size is estimated at 1,400 animals. We examined levels of variability at 16 microsatellite loci in elk from the transplanted and source populations. Overall, the populations differed significantly ($P < 0.0001$) in allelic and genotypic structure. Alaskan elk exhibited lower allelic diversity than elk from the Olympic Peninsula (3.0 vs. 3.7 alleles per locus, respectively), and the only monomorphic locus was observed in Alaskan elk. Individual Alaskan elk were heterozygous at significantly ($P = 0.002$) fewer loci than were Olympic Peninsula elk (40.6% vs. 52.0%, respectively), and the fixation index (f or F_{IS}), an indicator of the relative extent of inbreeding, was greater in Alaskan elk. The level of heterozygosity in Alaskan elk was less than expected based on a prediction related to the number of founders and genetic characteristics of the founding population, indicating a substantial effect of genetic drift. Possible mechanisms accounting for drift are poor representation of allelic diversity in the founders and/or poor survival among the founders, both of which must be considered critically in evaluating the potential success of future transplants.

Appendix 2. Abstracts of publications:

Variation in mtDNA haplotypes from moose in Alaska and northwestern British Columbia: taxonomic and management implications

Kris J. Hundertmark, R. Terry Bowyer, Gerald F. Shields, Charles C. Schwartz, and Michael H. Smith

K. J. Hundertmark.¹ Alaska Department of Fish and Game, Kenai Moose Research Center, Soldotna, AK, USA 99669, and Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, USA 99775

R. T. Bowyer and G. F. Shields. Department of Biology and Wildlife and Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, USA 99775 (present address of GFS: Dept. of Natural Sciences, Carroll College, Helena, MT 59601)

C. C. Schwartz. Alaska Department of Fish and Game, Kenai Moose Research Center, Soldotna, AK, USA 99669 (present address: Forestry Sciences Laboratory, Montana State University, Bozeman, MT 59717)

M. H. Smith. Savannah River Ecology Laboratory, Aiken, SC, USA, and Departments of Genetics, Forestry, and Ecology, University of Georgia, Athens GA

Abstract: We assessed the phylogeographic history of moose (*Alces alces*) in southeastern Alaska, USA, to determine their genetic affinity to surrounding populations and to clarify their origin and uncertain taxonomic status. Moose from southeastern Alaska were characterized by two sequences that were highly divergent from those in the remainder of the state; overlap occurred only in the northernmost section of the southeastern panhandle. Moose inhabiting British Columbia, Canada, showed high haplotype diversity, which they shared with moose in southeastern and interior Alaska, in addition to having haplotypes that were restricted to that area. Similarity between geographic distribution and phylogenetic structure of haplotypes indicates temporal and spatial separation of moose in the past. We hypothesize that moose representing an early split from the colonizing wave in the late

Pleistocene and early Holocene colonized most major drainages of southeastern Alaska. Other lineages in British Columbia likely belong to a subsequent invasion of moose colonizing from the southeast. Coastal populations of moose living south of 58° 45' N latitude in southeastern Alaska should not be classified as *A. a. gigas*. Behavioral and morphological differences between *A. a. gigas* and other forest-dwelling subspecies in North America indicate the need to reevaluate management practices in southeastern Alaska.

Genetic Relationships Deduced From Cytochrome-*b* Sequences among Moose

Kris J. Hundertmark^{1,2}, Gerald F. Shields^{2,3}, R. Terry Bowyer², and Charles C. Schwartz^{1,4}

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ABSTRACT: We studied variation in nucleotide sequences of the mitochondrial cytochrome-*b* gene to assess the phylogeny of moose (*Alces alces*) in general, and the position of North American moose within that phylogeny in particular. We combined North American, Asian, and European haplotypes generated for this study with 3 Eurasian haplotypes obtained from GenBank. No nucleotide variation occurred within moose from North America, whereas 3 haplotypes were present in European moose and 4 haplotypes in Asian moose. Clade structure was consistent over 6 most-parsimonious trees, with Asian haplotypes composing 1 clade, and North American and European haplotypes composing a second, albeit poorly supported clade. Low diversity of nucleotides in cytochrome *b* indicated a recent ancestry among moose worldwide. Existence of 1 North American haplotype is strong evidence of a single, recent entry into the New World via the Bering land bridge, rather than multiple entries through ≥ 1 corridors. Furthermore, no phylogenetic support existed for the theory of distinct lineages of European versus Asian-North American moose.

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Mitochondrial Phylogeography of Moose (*Alces Alces*) in North America

Kris J. Hundertmark,* R. Terry Bowyer, Gerald F. Shields, and Charles C. Schwartz

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Nucleotide variation was assessed from the mitochondrial control region of North American moose (*Alces alces*) to test predictions of a model of range expansion by stepping-stone dispersal, and to determine if patterns of genetic variation support the current recognition of 4 subspecies. Haplotypes formed a star phylogeny indicative of a recent expansion of populations. Values of nucleotide and haplotype diversity were low continent-wide, but were greatest in the central part of the continent and lowest in peripheral populations. Despite low mitochondrial diversity, moose exhibited a high degree of differentiation regionally, which was not explained through isolation-by-distance. Our data indicate a pattern of colonization consistent with a large central population that supplied founders to peripheral populations (other than Alaska), perhaps through rare, long-distance dispersal events (leptokurtic dispersal) rather than mass dispersal via a stepping-stone model. The colonization scenario does not account for the low haplotype diversity observed in Alaska, which may be derived from a post-colonization bottleneck. Establishment of peripheral populations by leptokurtic dispersal and subsequent local adaptation may have been sufficient for development of morphological differentiation among extant subspecies.

Mitochondrial Phylogeography of Moose (*Alces alces*): Late Pleistocene Divergence and Population Expansion

Kris J. Hundertmark,^{*†1} Gerald F. Shields,^{†‡} Irina G. Udina,[§] R. Terry Bowyer,[†] and Charles C. Schwartz^{*¶}

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Sequence variation within the left hypervariable domain of the mitochondrial control region of moose (*Alces alces*) occurred at low to moderate levels worldwide, and was structured geographically. Partitioning of genetic variance among regions suggested isolation-by-distance as the primary agent for differentiation of moose, and does not support the existence of distinct eastern and western races. Levels of genetic variation and structure of phylogenetic trees indicate Asia as the origin of all extant lineages. A recent coalescence is indicated, with the common mitochondrial ancestor dating to the last ice age. Moose have undergone two episodes of population expansion, likely corresponding to the final interstade of the most recent ice age and the onset of the current interglacial. Timing of expansion for the population in the Yakutia-Manchuria region of eastern Asia indicates that it is one of the oldest populations of moose, and may represent the source of founders of extant populations in North America, which were colonized within the last 15,000 years. The population in Magadan Oblast, Russia, adjacent to the Bering Sea, expanded recently and was not related closely to moose in North America, indicating colonization of that area subsequent to the colonization of the New World. Our data suggest an extended period of low population size or a severe bottleneck prior to the divergence and expansion of extant lineages, and a recent, less-severe bottleneck in Europe. Climate change during the last ice age, acting through contraction and expansion of moose habitat and the flooding of the Bering land bridge, undoubtedly was a key factor influencing the divergence and expansion of moose populations.

Appendix 3. Memorandum describing an analysis of the literature relevant to conservation genetics of Cook Inlet beluga whales.



MEMORANDUM

ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
43961 KALIFORNKY BEACH ROAD, SUITE B
SOLDOTNA, ALASKA 99669

(907) 262-9368

To: Don Spalinger
Research Coordinator
Wildlife Conservation
Region II

Date: 30 July 2001

Thru:

From: Kris J. Hundertmark
Wildlife Biologist III
Soldotna

Subject: Beluga genetics and endangered
species classification

Last year, the Alaska Chapter of The Wildlife Society submitted a document to Wayne Regelin outlining reasons why the Cook Inlet population of beluga whales should be listed as an endangered subspecies under the Endangered Species Act. Part of the justification for that opinion was a published report (O'Corry-Crowe et al. 1997) documenting the distinct genetic status of that population. O'Corry-Crowe et al. (1997) examined variation in mitochondrial DNA (mtDNA) among populations of belugas in Alaska and northwestern Canada. They measured genetic relationships among those populations and found that the Cook Inlet population was the most distinctive (i.e., had the least amount of genetic relatedness to other populations). That was an interesting and informative study, but it only told half of the story; therefore, I believe the TWS call for endangered species status for Cook Inlet belugas was premature, primarily because it was based largely on limited genetic data.

mtDNA is a useful genetic marker for addressing historic demographics and biogeographic patterns of populations. It is maternally inherited and does not suffer from the confounding effects of recombination; thus, it is a very powerful tool for tracing maternal lineages through time and space. It is not, however, the marker of choice when examining topics such as exchange of individuals between adjacent populations over short periods of time. Nuclear markers (e.g. allozymes, microsatellites, minisatellites, SNPs. etc.) offer a much more complete

picture for examining gene flow among populations over the short term. Theoretically, it takes only a few migrants per generation to keep two populations from diverging. In species that exhibit strong female philopatry, it is male-mediated gene flow that is the important factor in maintaining genetic continuity among populations. That is a primary example why genetic-based studies of population identity are superior to telemetry-based studies. Evolutionarily significant levels of population mixing can occur at temporal scales and with migration rates that are virtually undetectable with marked animals.

Recent publications have documented the different outcomes of mitochondrial versus nuclear studies of the same population. We should all be familiar with the unique genetic status applied to the ABC brown bears as a result of the mtDNA study of Talbot and Shields (1996). A subsequent examination of nuclear markers, however, documented no restriction in gene flow among the ABC islands and the adjacent mainland (Paetkau et al. 1998). Furthermore, analysis of mtDNA from fossil brown bears (Leonard et al. 2000) indicated that the phylogeographic structure of Alaskan bears was a relatively recent development rather than an ancient one as proposed by Talbot and Shields (1996).

Closer to home, a recent publication examined mitochondrial and nuclear genetic variation in Arctic Ocean belugas (Brown Gladden et al. 1999), and demonstrated that those markers are informative at different scales. Belugas from the Bering Sea to the Gulf of St. Lawrence were segregated into 9 geographic populations based on mitochondrial data, but only 2 groups based on nuclear data. The reason given for that difference was the population mixing (male-mediated) that occurs on winter ranges. Thus, distinct populations based on mitochondrial data alone actually were not distinct when looking at the entire genome. The authors used their results to identify evolutionarily significant units (ESUs) based on nuclear data and management units based on mitochondrial data. Although that publication did not sample Cook Inlet belugas, it raised the concern that mtDNA data alone are not sufficient to deduce population status under the ESA. Unfortunately, the TWS position paper did not cite that study even though it was available at the time. It also is not listed in the references of the petition for endangered status presented to NMFS by the Center for Biological Diversity, although O'Corry-Crowe et al. (1997) is.

It is not my intention to disparage a mostly well-written and -researched position paper. It is entirely possible that Cook Inlet belugas deserve protection under the ESA, and nuclear genetic data may support the unique status of that population. But I believe that ESU designation for Cook Inlet belugas is premature based only on the mitochondrial genetic data. For such an important issue, nuclear data must be considered.

Literature Cited

Brown Gladden, J. G., M. M. Ferguson, M. K. Friesen, and J. W. Clayton. 1999. Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA variation. *Mol. Ecol.* 8:347-363.

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Paetkau, D., G. F. Shields, and C. Strobeck. 1998. Gene flow between insular, coastal and interior populations of brown bears in Alaska. Mol. Ecol. 7:1283-1292.

Talbot, S. L., and G. F. Shields. 1996. Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. Mol. Phyl. Evol. 5:477-494.

cc: J. Hughes

VII. PROJECT COSTS FOR THIS SEGMENT PERIOD

FEDERAL AID SHARE \$ 11,932 + STATE SHARE \$ 3,977 = TOTAL \$15,909

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APPROVAL DATE: _____